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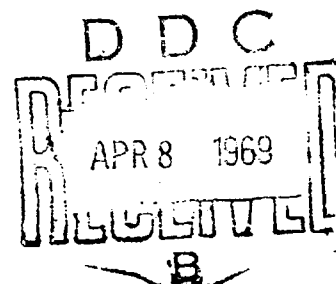
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STUDIES ON LEPTOSPIROSIS IN THAILAND, WITH SPECIAL REFERENCE
TO THE EPIDEMIOLOGY, PATHOLOGY AND CLINICAL ASPECTS,
AND ITS RELATION TO THE ANIMAL RESERVOIR HOSTS.

by

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THE BANGKOK SCHOOL OF TROPICAL MEDICINE
UNIVERSITY OF MEDICAL SCIENCES

FINAL PROGRESS REPORT NO. IV.
(THE FOURTH YEAR)

STUDIES ON LEPTOSPIROSIS IN THAILAND,
WITH SPECIAL REFERENCE TO THE EPIDEMIOLOGY,
PATHOLOGY AND CLINICAL ASPECTS, AND ITS RELATION.
TO THE ANIMAL RESERVOIR HOSTS.

GRANT NUMBER DA-CRD-AFE-S92-544-68-G107
PERIOD COVERED 1 January 1968 - 31 December 1968.

FINAL PROGRESS REPORT NO. IV.

Research Grant No. :

DA-CRD-AFE-S92-544-68-G107

Title of the Project:

Studies on leptospirosis in Thailand,
with special reference to the epidemiology,
pathology and clinical aspects, and its
relation to the animal reservoir hosts.

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Period covered:

1 January 1968 - 31 December 1968.

ABSTRACT

Epidemiological study of suspected cases of leptospirosis in 39 provincial hospitals in Thailand during January 1968 - December 1968 revealed 197 positives out of 1,377 cases (14.3%). Common Serogroups were L.icterohemorrhagiae, L.autumnalis and L.wolffii. However, in Bangkok Hospitals leptospirosis batavia was common.

Annual variation of human leptospirosis in Thailand (residual antibodies survey) was studied for the third consecutive year and the results indicated that the incidence was slightly increased (from 4% to be 6% and 9% respectively.)

Surveys of leptospiral antibodies in Umong Canton (an endemic area near Chiangmai Province) revealed that the area was still considered to be an endemic area of leptospirosis.

The correlation studies at Pitsanuloke Province enabled us to find out an endemic area and revealed five leptospiral serogroups were prevalent in Pitsanuloke Province.

The dried blood filter paper method was experimentally studied and the results revealed that it was reliable and practical for diagnosis of the disease in suspected cases from provincial areas.

In animal experiments, L.javanica (Bangkok) L.icterohemorrhagiae (Uttaradith) and L.akiyami A (Uthaidanee) were all proved to be of high virulence to hamsters.

There were at least 20 serotypes of pathogenic leptospirae so far discovered from Thailand and belong to 12 serogroups.

STUDIES ON LEPTOSPIROSIS IN THAILAND

A. Human Leptospirosis

1. The incidence of leptospirosis in suspected cases in the Bangkok Area.
2. The incidence of leptospirosis in suspected cases in the Provincial Areas.
3. Annual variation of human leptospirosis in Thailand (continued).
4. Surveys of leptospiral antibodies in an endemic area of Chiangmai Province.
5. Studies on correlation between human and animal leptospirosis.
6. Histopathological Studies in Leptospiral Hepatitis.

B. Leptospirosis in Animals

7. Leptospirosis in rats, dogs and swine in five different parts of Thailand.

C. Laboratory and Experimental Studies

8. Pathogenesis of Leptospirosis javanica, icterohemorrhagiae and akiyami A.
9. A comparative serological investigation for the use of filter paper as a transport medium.
10. Pathogenic leptospirae discovered from Thailand.

A. HUMAN LEPTOSPIROSIS

1. THE INCIDENCE OF LEPTOSPIROSIS IN SUSPECTED CASES OF BANGKOK AREA.

During the past year (1 January - 31 December 1968)

hemocultures and serological determinations in suspected cases from 10 hospitals in Bangkok revealed the following results:

(1) Hemoculture

Hemocultures were performed in 41 cases with three positive results. The causative organisms were L. bataviae in two cases and L. javanica in one.

(2) Serological studies

Agglutination tests were performed in 514 specimens (432 cases) with 37 positive results. The serogroups involved were:-
22 L. bataviae, 5 L. javanica, 3 L. australis, 2 L. canicola, 1 L. autumnalis, 1 L. hebdomadis, 1 L. icterohemorrhagiae, 1 L. pomona and 1 L. pyrogenes.

Summary and conclusion

In comparison with the last year, the numbers of positive hemocultures and agglutinations from suspected cases of leptospirosis in the Bangkok area were slightly decreased. The serogroups involved in both periods were almost the same. L. bataviae was the most prevalent serogroup in the Bangkok area responsible for leptospirosis in man.

2. SEROLOGICAL STUDIES OF SUSPECTED CASES OF LEPTO--
SPIROSIS IN PROVINCIAL HOSPITALS BY DRIED BLOOD ON
FILTER PAPER; A FOURTH YEAR RECORD.

During the past twelve months (January-December 1968), 1,642 specimens of dried blood on filter paper (1,377 cases) from 39 provincial hospitals in Thailand and some hospitals in the Philippines and Singapore, were sent to our laboratory for determination of leptospiral antibodies. Of the 1,377 suspected cases, 197 gave positive results (significant titres of over 1:100). The serogroups involved were: 44 L. icterohemorrhagiae, 39 L. autumnalis, 29 L. wolffii, 20 L. bataviae, 15 L. grippotyphosa, 15 L. hebdomadis, 11 L. australis, 11 L. pyrogenes, 7 L. javanica, 5 L. canicola and 1 L. pomona.

Summary and Conclusions.

Compared with last year, the number of specimens and positive cases decreased considerably. The most prevalent serogroup encountered during the year was L. icterohemorrhagiae, whereas in 1967 L. autumnalis predominated. L. wolffii was also more prevalent in the current year than in 1967.

3. ANNUAL VARIATION OF HUMAN LEPTOSPIROSIS IN THAILAND

From our previous report (Final Progress Report No. III) the variations in incidence from year to year were obvious. In 1964 the incidence was fairly high in all parts of the country (an average of 27%), but in the past two consecutive years of 1966 and 1967 the incidence were markedly decreased. (to be 4 and 6% respectively)

During 1968, we have continued our studies for surveying the residual antibodies in general population with the following results:

Surveys of 1968

Part	No. of provinces	No. examined	No. positive	% positive	Serogroup Involved
Central	10	1096	61	6	11I, 10B, 6Au, 6J, 5Ak, 5Py, 5W, 4C, 4G, 3Po, 2H
North	8	608	82	14	31W, 19I, 7Ak, 6B, 5J, 3H 3Hy, 3Py, 2Au, 2G, 1C
South	9	547	44	8	10B, 8I, 8W, 4Ak, 4G, 4Py 2C, 2H, 1J, 1Hy
N-east	7	393	34	9	10I, 7Au, 7G, 4Py, 2Ak, 1G 1H, 1J, 1W.
East	5	415	40	10	9I, 7H, 6Ak, 4Au, 4B, 3G, 3W, 2Po, 1Hy, 1Py
5 parts	39	3059	261	9	57I, 48W, 30B, 24Ak, 20C, 19Au, 17Py, 15H, 13J, 8C, 5Hy, 5Po.

Ak = akiyami A, Au = australis, B = bataviae, C = Canicola,

G = grippotyphosa, H = hebdomadis, Hy = hyos, I = icterohemorrhagiae, J = javanica, Po = pomona, Py = pyrogenes and W = wolffii.

To compare with the results of the past two years, the results of 1966 and 1967 were shown below:

Surveys of 1966

Part	No. of Provinces	No. examined	No. positive	% positive	Serogroup Involved
Central	10	571	16	3	5L, 2B, 2H, 2Py, 2W, 1C, 1G, 1J.
North	8	548	33	6	11I, 5Ak, 5G, 3Au, 3W, 2J, 1B, 1C, 1H, 1Py.
South	9	448	11	2	4Py, 3B, 2C, 1I, 1W.
N-east	7	550	12	2	7I, 2J, 1Au, 1Po, 1Py.
East	5	381	28	7	10B, 9I, 6H, 2W, 1Ak.
5 Parts	39	2487	100	4	33I, 16B, 9H, 8G, 8Py, 8W, 6Ak, 5J, 4Au, 2C, 1P.

Surveys of 1967

Part	No. of Provinces	No. examined	No. positive	% positive	Serogroup Involved
Central	10	686	30	4	10I, 4C, 4Py, 3B, 3W, 1Ak, 1Au, 1G, 1H, 1My, 1P.
North	8	616	37	6	8I, 4H, 4Hy, 4J, 4W, 3B, 3C, 2Au, 2G, 2Py, 1Ak.
South	9	602	47	7	15I, 14W, 5G, 3B, 3C, 3H, 2J, 1Au, 1Py.
N-east	7	417	30	6	10I, 4H, 3Au, 3Py, 2B, 2C, 2J, 2W, 1Ak, 1Po.
East	5	274	21	6	5H, 5J, 3W, 2B, 2C, 2G, 1Hy, 1Py.
5 Parts	39	2715	165	6	48I, 26W, 17H, 14C, 13B, 11Py, 10G, 8J, 7Au, 6Hy, 3Ak, 2Po.

Discussion and Conclusion

From the results of our studies, the incidences of leptospirosis during the past three consecutive years (1966, 1967, 1968) were considered to be low as compare with the year 1964. The serogroups involved were also of interest. L.icterohemorrhagiae was the most common organisms throughout the past three years, and L.wolffii was markedly increased in the past two years (1967-1968). The reasons of the fluctuations of these incidences of leptospirosis are being sought out.

4. SURVEYS OF LEPTOSPIRAL ANTIBODIES IN AN ENDEMIC AREA OF CHIENGMAI PROVINCE (UMONG CANTON)

In the year 1965, we reported the findings of leptospiral antibodies among the general population of Chiengmai Province (North Thailand) and the villagers of Umong Canton which was considered to be an endemic area of leptospirosis. (J. of Med. Ass: of Thailand. 48, 223, 1965. The findings revealed that 37.0% of general population of Chiengmai gave positive reactions with their titers ranging from 1:100 to 1:3,000. L.icterohemorrhagiae was the most common serotype found in this province (most of the patients admitted into the Chiengmai Provincial Hospital were found also suffered from L.icterohemorrhagiae. From Umong Canton, an area nearby Chiengmai (10 miles away), the findings revealed 65.2% of the farmers were positive, most of which had the antibodies against L.grippotyphosa and L.javanica.

Two years later, we repeated our studies in Umong Canton and found that the incidence was decreased considerably. (The same as general survey in all parts of the country). The findings revealed only 16.6% of positive reactions among those farmers. Most of them had the antibodies against L. grippotyphosa and L. javanica which were the same serogroups obtained from the first survey.

In November 1968, we tried to study leptospirosis in Umong Canton again with the following results:

Out of 48 blood specimens examined, 13(27%) were positive with the titers ranged from 1:100 to 1:300. Of the total 13 positive cases, seven (53.8%) had the antibodies against L. javanica, four (30.8%) against L. icterohemorrhagiae, one (7.7%) against L. australis and L. grippotyphosa.

We have also studied in the general population of Chiangmai Province at the same period of time with the following results:

Out of 88 blood specimens examined, 7(8%) were positive. Six had the antibodies against L. wolffii and one had the antibodies against L. bataviae.

Discussion :

Our findings revealed that the incidence of positive leptospiral antibodies in Chiangmai people (8%) was markedly decreased in comparison with the first survey (37%). The leptospiral serogroups

involved were also different. Among the farmers of Umong Canton the incidence was also marked decreased in comparison with the first survey. (from 65.2% to be 27%). However, the third survey was slightly increased in comparison with the second survey (from 17% to be 27%). L. javanica was still the most common serogroup found in such area, while L. grippotyphosa was markedly decreased.

Conclusion:

Leptospiral antibodies survey in Chiengmai Province and Umong Canton were again studied. The results indicated that the incidences of leptospirosis both in general population of Chiengmai Province and in the endemic area were markedly decreased. The leptospiral serogroups involved in Chiengmai Province were also different from the first survey. Umong Canton was still considered to be an endemic area of leptospirosis, the incidence of which being 27% as compare with that of 8% in downtown area of Chiengmai

5. STUDIES ON CORRELATION BETWEEN HUMAN AND ANIMAL
LEPTOSPIROSIS AT PITSANULOK PROVINCE

Pitsanulok Province is about two hundred and fifty miles up north from Bangkok City. From July to October in 1959, over twenty cases of leptospirosis were reported from Budhachinaraj Hospital of that province, and in the year 1966 over sixty cases were reported again. In 1967 our team started investigation on leptospirosis in rats, dogs and swine of Pitsanulok Province, and found a fairly high incidence among the animals of that province. During September and November 1968 we studied on the correlation between human and animal leptospirosis in Pitsanuloke Province, using Budhachinaraj Hospital as a center. The studies included the followings:

1. Hemocultures and serological examinations in all cases of pyrexia of unknown origin both at the out-patient clinic and admitted cases.
2. Follow up to the positive cases' residential areas to study residual antibodies in apparently normal healthy people and also serological studies in the dogs of the same areas.
3. Survey of residual leptospiral antibodies among general population of Pitsanulok Province.

Results

(1) Hemocultures and serological studies

During two months at the out patient clinic and in the medical wards of Budhachinaraj Hospital, hemocultures were performed in 104 cases of pyrexia of unknown origin. Four specimens were positive with the following serogroups: two L. autumnalis, one L. bataviae and one L. grippotyphosa.

Serological determinations of 25 paired sera (in 25 cases out of a total of 104 cases examined) yielded four positive rising agglutinin titre cases, the serogroups of which were two L. hebdomadis, one L. autumnalis and one L. wolffii.

From a total of eight positive cases, one case was positive by hemoculture alone, four cases were positive by serological studies alone (rising agglutinin titres) and three cases were positive both hemoculture and serological confirmation.

From hemocultures and serological studies, the results revealed of at least five leptospiral serotypes were responsible for human leptospirosis in the area of Pitsanulok Province.

(2) Follow up to the positive cases' residential areas

According to the availability of communication and transportation, two cantons were visited. The results of preliminary investigation were as follows.

A. Wungnok-Ann Canton (the patient suffering from L. hebdomadis)

Fourty three normal healthy persons were examined serologically for residual antibodies with 13 positives (30.23%), details were set out in the table below:

Titre (Reciprocal)	100	300	1,000	3,000	10,000	30,000	Sub-Total
L. akiyami A	1	1	0	0	0	0	2
L. australis	0	0	0	0	0	0	0
L. bataviae	0	0	1	0	0	0	1
L. canicola	0	0	0	0	0	0	0
L. grippotyphosa	0	0	0	0	0	0	0
L. hebdomadis	2	0	0	0	0	1	3
L. hyos	1	0	0	0	0	0	1
L. ictero	2	0	1	0	0	0	3
L. javanica	1	0	0	0	0	0	1
L. pyrogenes	0	0	0	0	0	0	0
L. wolffii	2	0	0	0	0	0	0
Total	9	1	2	0	0	1	13

Preliminary results of examinations of the dogs in this Canton were as follows:-

Fourty dogs were examined serologically with 10 positives (25%). The serogroups involved were as follows: 4 L.icterohemorrhagiae, 3 L.hebdomadis one L.canicola, one L.grippotyphosa and one L.pyrogenes. All positive dogs revealed an agglutinin titre of 1:100.

B. Ban-Grang Canton (Two positive cases, one suffering from L.hebdomadis, and the other suffering from L.grippotyphosa).

Fourty four normal healthy persons were examined with six positives (13.6%), and all positive cases revealed an agglutinin titre of 1:100. The serogroups involved were as follows: 2 L.akiyami A, 2 L.pyrogenes, one L.hebdomadis and one L.javanica.

The blood of the dogs in the Canton was collected and examined for leptospirosis. The results are still in progress.

(3) Survey of residual leptospiral antibodies among general population of Pitsanulok Province.

451 specimens of blood were examined with 64 seropositives (14.2%). From 416 males with 61 positives (14.7%) and 35 females with 3 positives (8.6%). The following serogroups were involved: 19 L.icterohemorrhagiae, 17 L.akiyami A, 10 L.pyrogenes, 5 L.javanica, 5 L.grippotyphosa, 3 L.wolffii, one L.australis, one L.bataviae, one L.canicola, one L.hebdomadis, and one L.hyo.

Discussion and Conclusion:

Among the general population of Pitsanulok Province, the incidence of leptospirosis was about 15 percent (by serological examinations). At Wangnok-Ann Canton, the incidence was 30 percent. The common serogroup of leptospirae involved both in man and in dogs was L. hebdomadis. Thus Wangnok-Ann Canton may be considered as an endemic area of leptospirosis in Pitsanulok Province.

From our previous studies, the three serotypes of leptospirae isolated from rats of Pitsanulok Province were L. akiyami A (most common) L. javanica and L. pyrogenes. The present study demonstrates that there are many more serogroups of leptospirae in this province, and the animal reservoir hosts should be thoroughly studied in the future.

6. HISTOPATHOLOGICAL STUDIES IN LEPTOSPIRAL HEPATITIS

During the past three years, we have performed liver biopsies in 35 cases of leptospiral hepatitis at Vajira Municipal Hospital. Thirty one males and four females with ages ranging from 17-49 years were included in the present series. The serogroups of causative organisms were as follows: L. bataviae, L. canicola, L. javanica, L. icterohemorrhagiae, L. akiyami A, L. pyrogenes, L. grippotyphosa and L. australis.

The followings were results of histopathological studies:

I. Leptospirosis bataviae (22 cases)

First week (nine specimens)

The liver architecture was normal. The portal tracts in most cases were normal, in some cases may show very slight inflammatory cellular reaction. The predominant cell type was polymorphonuclear leukocyte, lymphocyte and eosinophil were also seen in a certain cases. Limiting plates were intact and most of the sinusoids were also normal. Kupffer cells showed hypertrophy and phagocytosis (r. b. c.) Intralobular bile duct was normal.

Second week (eleven specimens)

The liver architecture was still preserved and the liver cells were enlarged. There were lytic and eosinophilic necroses associated with mononuclear cell reaction. In certain cases the eosinophilic necrosis may be extreme with the formation of acidophilic bodies. The number of binucleated cells was increased. The giant parenchymal cell may be seen. Accumulation of bile pigment may also be seen in the cytoplasm of parenchymal cells. The Kupffer cells were prominent with r. b. c. and bile pigment inside. There was congestion in most of central sinusoids. The central veins appeared normal. In only one specimen the liver appeared fatty metamorphosis (may be due to the previous damage of the liver).

Third and Fourth week (five specimens)

Liver architecture may be normal or some foci of reticulin collapse were seen. A mild degree of inflammatory cellular reaction was a constant finding. Most of the cells were mononuclear with occasionally seen of lymphocyte or eosinophil. Binucleated parenchymal cells may also be seen but were not numerous.

Fourth week (one specimen)

There were only minimal non specific changes of the liver.

II. Leptospirosis canicola (five cases).

First week (one specimen)

Only portal tracts showed very slight inflammatory reaction.

Second week (four specimens)

The liver architecture was well preserved. Both lytic and eosinophilic necroses were seen. Cellular reaction was composed of mononuclear, polymorphonuclear and eosinophil cells. Kupffer cells were prominent with iron and bile pigment inside. Accumulation of iron was also seen in the cytoplasm of parenchymal cells.

Third week (three specimens)

There was an increase in number of binucleated liver cells with a mild degree of inflammatory cellular reaction.

III. Leptospirosis javanica (two cases)

Second week (two specimens)

The liver architecture was preserved, and the liver cells were enlarged. Eosinophilic necrosis was present. Bile stasis appeared in a diffuse fine granular form in the liver cytoplasm. Cellular infiltration was seen and most of the cells were mononuclear. Eosinophils and lymphocytes were also occasionally seen. Iron and bile pigment were seen in the Kupffer cells.

Fourth week (one specimen)

The liver architecture was slightly changed. Only congestion and cloudy swelling of the liver cells were seen.

IV. Leptospirosis pyrogenes (one case)

This case, the biopsies were made during the second and the fifth week.

Second week

The liver architecture was preserved. The liver cells were enlarged with the irregularity of their sizes. Binucleated liver cells were seen. Kupffer cells were hypertrophy and showed phagocytosis. The liver cells showed marked changes, including ballooning of the parenchymatous with diffuse small greenish droplets in the cytoplasm. Bile thrombi in canaliculi and leaking of bile were also seen. (This case was classified clinically a severe case).

Fifth week

The lesion was almost subsided, only non specific minimal change was seen.

V. Leptospirosis akiyami A (two cases)

One case was studied twice, the first and the third week specimens. (Case No.1).

First week (Case No.1)

The liver architecture was preserved. Limiting plates were intact and the liver was congested. There were cellular infiltrations and most of the cells were polymorphonuclear and mononuclear leukocytes.

Third week (case No.1)

The liver architecture was also preserved. Liver was congested and the sizes of liver nuclei were varied. Slight accumulation of iron in the Kupffer cells were seen.

Second week (Case No.2)

The liver was congested and showed fatty metamorphosis. The liver architecture was well preserved with scattered cellular infiltration. Most of the infiltrated cells were mononuclear cell. The liver cells were enlarged and some showed multinucleated. The Kupffer cells were prominent and showed phagocytosis.

VI. Leptospirosis icterohemorrhagiae (one case)

Second week

The liver architecture was preserved. The liver cells showed congestion and irregularity in sizes. Foci of eosinophilic necrosis were present. There was a moderate degree of mononuclear cell infiltration around the portal area. Accumulation of iron and bile pigment were seen in the liver cell parenchyma. Binucleated cells were also present.

VII. Leptospirosis grippotyphosa (one case)

Second week

Only congestion and minimal changes were seen.

VIII. Leptospirosis australis A (one case)

Second week

The liver revealed edema and congestion. The liver architecture was still preserved. The number of binucleated cells was increased. The Kupffer cells were prominent. There was congestion in most of central sinusoids and the central vein revealed normal. No cellular infiltration was seen.

Conclusion

Histopathological changes of the liver in thirty five cases of human leptospirosis were studied. Twenty two cases suffered from

L. bataviae, five cases from L. canicola, two cases each from L. javanica and L. akiyami A, one case each from L. pyrogenes, L. icterohemorrhagiae, L. grippotyphosa and L. australis A. The histopathologic changes in our series were not seriously impaired. During the fourth and the fifth week, the changes were very slight. The liver functions in most cases returned to normal by the end of the third week and during the fourth week.

7. LEPTOSPIROSIS IN RATS, DOGS AND SWINE IN FIVE MAJOR PARTS OF THAILAND.

In the Final Progress Report No. I, we showed the results of the investigations on the animal leptospirosis in many provinces of five major parts of Thailand, and since then the work has been continuously carried on. The summary of all of the results is as follows:-

CENTRAL AREA

(1) Rats

No.	Province	No. of rats	Species* of rats	No. positive		Serotyping of the isolates
				Culture	Sero	
1.	Bangkok	1,252	5	537/1252	338/903	439 <u>bataviae</u> , 97 <u>javanica</u> and 1 <u>akiyami A</u>
2.	Ayudya	150	4	19/143	8/150	16 <u>javanica</u> , 2 <u>bataviae</u> and 1 <u>pyrogenes</u>
3.	Saraburi	110	6	13/110	1/109	11 <u>bataviae</u> , 1 <u>hebdomadis</u> and 1 <u>pyrogenes</u>
4.	Rajburi	98	6	38/92	17/87	27 <u>bataviae</u> , 10 <u>javanica</u> and 2 <u>pomona</u>
5.	Petchburi	91	6	13/87	7/85	12 <u>bataviae</u> and 1 <u>javanica</u>
6.	Kanchanaburi	75	6	6/72	11/75	6 <u>akiyami A</u>
7.	Supanburi	75	5	42/68	13/75	25 <u>bataviae</u> 10 <u>akiyami A</u> and 7 <u>javanica</u>
8.	Singhburi	131	4	52/125	15/130	32 <u>bataviae</u> , 18 <u>javanica</u> and 2 <u>akiyami A</u>
9.	Nontburi	71	3	9/69	6/69	6 <u>bataviae</u> and 3 <u>javanica</u>
10.	Smutsakon	103	4	43/103	24/100	38 <u>bataviae</u> and 5 <u>javanica</u>
11.	Nakonsawan	49	4	1/49	1/45	1 <u>javanica</u>
12.	Uthaidani	68	5	3/68	5/68	2 <u>akiyami A</u> and 1 <u>javanica</u>

* Species of rats : 1,356 R. norvegicus, 300 R. rattus, 192 B. indica,
145 R. exulans, 88 Mus. musculus, 87 Sancus murinus, 100 B. bengalensis,
4 berdmorei. and 1 R. rajah.

Summary and Conclusion

From 2,273 rats trapped from 12 provinces of central Thailand (12 out of 19 provinces), R. norvegicus was the most prevalent rat species trapped from the down town area. Among 776 isolates obtained from kidney cultures, the organisms were identified as 592 L. bataviae, 159 L. javanica, 21 L. akiyami A., 2 L. pyrogenes, 1 L. hebdomadis and 1 L. pomona. The higher percentage of isolations (776 out of 2138) in comparison with lower percentage of serological positives (446 out of 1898) may indicate that the rats in Central Thailand are potential carrier hosts of leptospirosis.

(2) Dogs

No.	Province	No. exam.	Sero. pos. dogs		Serogroup Involved**
			No.	%	
1	Bangkok*	1157	632	54.6	420B, 16J, 24C, 8Ak, 7I, and 6Py.
2	Ayuthya	67	13	19.3	12C and 1B.
3	Saraburi	73	15	20.0	8C, 5 I, and 2Py.
4	Rajburi	101	12	11.9	11B and 1C
5	Petchburi	61	10	16.4	2C, 2J, 2Py, 2W, 1H, and 1 I.
6	Kanchanaburi	105	21	20.0	16C and 5 Py.
7	Supanburi	62	7	11.3	3C, 2 I, 1G and 1Po.
8	Singhburi	72	4	5.6	3C and 1B.
9	Nonthburi	42	20	47.6	18C, 1 I and 1Py.
10	Samut-akon	62	14	22.6	7J, 5B, 1C and 1G.
11	Nakonsawan	80	18	22.5	16C, 1 I, and 1 Py.
12	Uthaidhani	83	8	9.6	5C, 1australis, 1 I, and 1 wolffi

* In Bangkok City, kidney cultures were performed in 163 dogs with 13 positives (8%).

** Ak = akiyami A, B = bataviae, C = canicola,
I = icterohemorrhagiae, J = javanica, Py = pyrogenes.

Summary and Conclusion

In Bangkok City and the nearby Nonthburi Province, the incidence of canine leptospirosis was considered to be high (over 40 per cent). L. bataviae was the most common serotype involved in Bangkok area which was coincided with leptospirosis in rats of the same area. Outside Bangkok City, L. canicola was the most common serotype detected among the dogs. The low percentage of isolation in comparison with the high serological positive among the dogs in Bangkok area (8% to 54.6%) indicated that the dogs in Bangkok were not potential carrier hosts.

(3) Swine

The swine killed at the Abattoir of Bangkok City were obtained from many provinces of Central Thailand. Isolations of leptospirae from kidney cultures of the swine killed at the Bangkok Abattoir were performed. Four out of 219 animals were positive (1.8%). All isolates were identified as L. pomona.

The details of serologic studies are as follows:

No.	Province	No. exam.	Sero positive		Serogroup Involved
			No.	%	
1	Ayuthya	51	3	6.0	1 Ak, 1 C and 1 J.
2	Saraburi	56	2	3.6	2 Ak.
3	Rajburi	108	5	4.6	3 B, 1 Po and 1 C
4	Petchburi	47	0	0	0
5	Kanchnaburi	48	3	6.2	2 H and 1 Po
6	Supanburi	34	2	6.0	2 Po.
7	Singhburi	8	0	0	0
8	Samutsakon	22	2	9.0	2 B.
9	Uthaidhani	42	2	4.8	1 Po and 1 C.

Summary and Conclusion

The incidence of leptospirosis in swine of Central Thailand was considered to be low. The common serogroups involved were L. bataviae and L. pomona. Isolations of the organisms from kidney cultures in the swine of the Bangkok Abattoir was successful in only 1.8 percent, thus the swine should be considered as insignificant carrier host of leptospirosis in Central Thailand.

II. North Thailand

(1) Rats

No.	Province	No. of rats	Species* of rats	No. positive		Serotyping of isolates
				Culture	Serolo.**	
1	Pitsanulok	293	5	61/279	15/177	50 <u>L. akyamiA</u> , 3 <u>L. javanica</u> , 1 <u>L. pyrogenes</u> and two were lost.
2	Sukhothai	174	4	16/144	2/174	14 <u>L. akyamiA</u> , 1 <u>L. hebdomadis</u> and 1 <u>L. javanica</u> .
3	Chiengmai	123	3	32/123	-	24 <u>L. javanica</u> , 1 <u>L. hebdomadis</u> and seven were lost.
4	Uttaradit	116	7	9/112	5/115	8 <u>L. akyamiA</u> and 1 <u>L. ictero-</u> <u>hemorrhagiae</u> .
5	Prae	73	4	3/71	1/73	all javanica
6	Nan	96	4	2/92	2/96	1 <u>L. javanica</u> and 1 <u>L. wolffii</u>
7	Lampang	48	4	0/47	1/47	0

* 453 B. indica, 224 R. rattus sp., 114 B. bengalensis, 65 R. exulans,
43 Mus musculus, 16 R. rajah, 6 R. sabanus, 1 R. berdmorci and
1 R. bowersi.

• = not done.

Summary and Conclusion

In the provinces of North Thailand, no Rattus norvegicus was trapped. Bandicota (field rats) were most common in all provinces. L. akiyami A and L. javanica were most prevalent among the rats of those provinces. The results showed fairly high percentage of positive kidney cultures as compare with low serological positives. Thus the rats of northern provinces were considered as potential carrier hosts.

(2) Dogs

No.	Province	No. exam.	Seropositive dogs		Serogroup Involved*
			No.	%	
1	Pitsanulok	100	18	18	16C and 2Py.
2	Sukhothai	100	13	13	7C, 3Py, 1Ak and 1J.
3	Chiengmai	51	29	56.8	13 I, 7H, 5C, 2B, and 2J.
4	Uttaradit	81	14	17.3	12C, 1Py and 1Ak.
5	Prae	84	15	17.8	12C, 2G, and 1 Hy.
6	Nan	87	5	5.7	2W, 1C, 1Ak and 1J.
7	Lampang	79	13	16.5	8W, 4C, and 1 G.

* Ak = akiyami A, B = batavinae, C = canicola, G = grippotyphosa,

H = hebdornadis, Hy = hyos, I = icterohemorrhagiae,

J = javanica, Py = pyrogenes, W = wolffii.

Summary and Conclusion

The incidence of canine leptospirosis in the northern part of Thailand was considered to be high only in Chiangmai Province. The most common serogroup involved in many provinces was L. canicola. From Chiangmai survey in 1965, L. icterohemorrhagiae was commonly found and it was coincided with those human leptospirosis at the same period, Leptospirosis icterohemorrhagiae being the most common infection found in Chiangmai Hospital.

(3) Swine

No.	Province	No. exam.	Seropositive		Serogroup Involved.
			No.	%	
1	Pitsanulok	100	1	1	1Po
2	Sukhothai	20	0	0	0
3	Uttaradit	61	9	14.7	7Po, 1B and 1 I.
4	Prae	71	9	12.7	3B, 2 I, 1H, 1G, 1Po, and 1J.
5	Nan	60	4	6.7	2 I, 1G. and 1B.
6	Lampang	72	13	16.5	8W, 4C and 1G.

Summary and Conclusion

Leptospirosis in swine of the northern provinces was considered to be low. L. pomona and L. wolffii were the most common serogroups involved.

III. Northeast Thailand

(1) Rats

No.	Province	No. of rats	Species* of rats	No. positive		Serotyping of isolates
				Culture	Serology	
1	Nakonrajsima	88	3	6/85	0/83	All <u>L. javanica</u>
2	Chaipum	90	5	2/87	0/86	All <u>L. akiyami</u> A.
3	Khon Khaen	441	5	80/378	19/441	66 <u>L. javanica</u> , 11 <u>L. pyrogenes</u> , and 3 <u>L. australis</u>
4	Udonrthani	102	3	9/87	1/102	All <u>L. javanica</u>

* 560 R. rattus, 63 R. exulans, 56 B. indica, 28 R. rajah, 7 B. bengalensis and 7 R. berdmorei.

Summary and Conclusion

In four out of fifteen provinces of the Northeast Thailand, the incidence of leptospirosis in rats was considered to be low with the exception of Khon Khaen province. The most prevalent serotype was L. javanica, and R. rattus was the most common rat species trapped in Northeast Thailand.

(2) Dogs

No.	Province	No. exam.	No. pos. dogs.		Serogroup Involved**
			No.	%	
1	Nakonrajsima	60	0	0	0
2	Chaipum	60	6	10	3 I, 2J, and 1 Hy.
3	Khonkan	150	6	4	2 I, 2C, 1Au and 1J.
4	Udonthani	80	1	1.7	1C.

** Au = australis, C = canicola, I = icterohemorrhagiae,
J = javanica and Hy = hyos.

Summary and Conclusion

The incidence of canine leptospirosis in the four northeast provinces was considered to be very low in comparison with the central and northern areas of Thailand.

IV. East Thailand

(1) Rats

No.	Province	No. of rats	Species* of rats	No. positive		Serotyping of the isolates
				Culture	serology	
1	Cholburi	238	6	44/221	38/163	38 <u>bataviae</u> , 3 <u>pyrogenes</u> , 1 <u>grippotyphosa</u> , 1 <u>icterohemorrhagiae</u> and 1 <u>javanica</u>
2	Rayong	93	4	1/81	2/93	<u>javanica</u>
3	Chanburi	130	6	48/112	17/121	40 <u>bataviae</u> , 4 <u>javanica</u> , 2 <u>akiyami A</u> , 1 <u>hebdomadis</u> and 1 <u>sentot</u> .
4	Trad	139	5	11/68	8/139	7 <u>javanica</u> and 4 <u>akiyami A</u> .
5	Nakonnayok	580	8	58/467	37/580	26 <u>javanica</u> , 13 <u>bataviae</u> , 7 <u>hebdomadis</u> , 4 <u>hyos</u> , 3 <u>akiyami A</u> , 2 <u>wolffii</u> , 1 <u>pyrogenes</u> , 1 <u>icterohemorrhagiae</u> and 1 <u>sentot</u>
6	Prachinburi	83	5	58/62	10/83	All <u>bataviae</u>

* 650 R. rattus sp., 199 R. norvegicus, 130 B. indica, 101 B. bengalensis,
59 R. berdmorei, 52 R. exulans, 50 R. rajah, 19 Sancus murinus,
and 3 R. sabanus.

Summary and Conclusion

In East Thailand, the incidence of leptospirosis in rats of Cholburi and Chanburi provinces was considered to be high. In other four provinces, the incidences were low. R. norvegicus of downtown areas harboured high percentage of L. bataviae which was almost the same as found in Bangkok city. Nine serotypes of pathogenic leptospirae were isolated from Nakhonnayok province.

(2) Dogs

No.	Province	No. exam.	Sero. pos. dogs		Serogroup Involved**
			No.	%	
1	Cholburi	112	26	23.2	9B, 7G, 6 I, 3H and 1Au.
2	Rayong	51	9	17.6	9 I.
3	Chanburi	75	12	16.0	4B, 4 I, 2C and 2H.
4	Trad	100	13	13.0	5B, 4Au, 2 I and 2J.
5	Nakhonnayok	81	37	45.6	18 I, 7H, 3G, 3Ak, 2C, 2Po, 1B and 1Py.
6	Prachinburi	51	12	23.5	4B, 3 I, 3H, 1G and 1 Au.

** Ak = akiyami A, Au = australis, B = bataviae, C = canicola,
 G = grippotyphosa, H = hebdomadis, I = icterohemorrhagiae,
 J = javanica, Po = pomona, Py = pyrogenes.

Summary and Conclusion

The most common serogroups of leptospirae in the dogs of East Thailand were found to be L. icterohemorrhagiae and L. bataviae.

(3) Swine

No.	Province	No. exam.	Seropositive		Serogroup Involved
			No.	%	
1	Rayong	13	0	0	0
2	Chanburi	73	4	5.5	3 <u>bataviae</u> and 1 <u>hyos</u>
3	Trad	27	0	0	0
4	Prachinburi	33	4	12.1	3 <u>bataviae</u> and 1 <u>ictero-hemorrhagiae</u>

Summary and Conclusion

The incidence of leptospirosis in the swine of East Thailand was considered to be very low.

V. South Thailand

(1) Rats

No.	Province	No. of rats	Species* of rats	No. positive		Serotyping of the isolates
				Culture	Sero.	
1	Chumpon	109	4	22/92	12/109	15 <u>L.icterohemorrhagiae</u> , 6 <u>L.javanica</u> and one was lost.
2	Ranong	96	6	20/72	6/96	15 <u>L.javanica</u> , 2 <u>L.pyrogenes</u> , 2 <u>L.icterohemorrhagiae</u> and 1 <u>L.canicola</u> .
3	Surathani	108	4	37/105	10/99	27 <u>L.bataviae</u> , 10 <u>L.javanica</u> .
4	Nakonsithamraj	133	5	28/132	13/131	18 <u>L.bataviae</u> , 5 <u>L.icterohemorrhagiae</u> , and 5 <u>L.javanica</u> .

* 173 R.norvegicus, 131 R.rattus, 71 Sancus murinus, 42 R.exulans, 11 R.rajah, 7 R.berdmorei and 5 B.indica.

Summary and Conclusion

The incidence of leptospirosis in the rats of four provinces of South Thailand was considered to be moderately high. L.bataviae, L.icterohemorrhagiae and L.javanica were the most common serotypes isolated.

(2) Dogs

No.	Province	No. exam.	Sero. pos. dogs		Serogroup Involved**
			No.	%	
1	Chumpon	63	10	15.8	4C, 1L, 1Ak and 1H.
2	Ranong	100	6	6	4W, 1J, and 1Py.
3	Surathani	100	12	12	7B, 3J, 1Hy, and 1W.
4	Nakonsithamraj	100	10	10	9C, and 1 I.

Summary and Conclusion

The incidence of canine leptospirosis in South Thailand was considered to be low and L. canicola was the most prevalent serogroup involved.

(3) Swine

No.	Province	No. exam.	Sero. pos. dogs.		Serogroup Involved**
			No.	%	
1	Chumpon	77	6	7.8	2Ak, 2J, 1Po and 1Au.
2	Ranong	87	7	8.0	3Po, 3Py and 1W
3	Nakornsitham- raj.	21	3	14.3	1Po, 1B and 1 I.

** Ak = akiyami A., Au = australis, B = bataviac, C = canicola,
H = hebdomadis, I = icterohemorrhagiae, J = javanica,
Po = pomona, Py = pyrogenes, W = wolffii.

Summary and Conclusion

The incidence of leptospirosis in the swine of South Thailand was also considered to be low.

C. LABORATORY AND EXPERIMENTAL STUDIES

8. Pathogenesis of Leptospirosis javanica, icterohemorrhagiae (Uttaradith strain) and akiyami A (Uthaidhanee strain) in experimental hamsters,

To correlate the findings in human leptospirosis javanica we then studied this serotype in animal experiments, and as we have mentioned in our final progress report No. III (January 1968) that the human Leptospirosis icterohemorrhagiae of Chiangmai was considered to be severe in both clinical manifestations and mortality rate. Unfortunately, last year we had no such isolate from Chiangmai Province and we have tried L. icterohemorrhagiae from Nakorn Srithamraj province of South Thailand. This year we could not isolate from Chiangmai Province but we could isolate L. icterohemorrhagiae from Uttaradith Province of North Thailand. This province is not very far from Chiangmai Province, we then tried again to study the pathogenesis of such isolate. Further more, we also studied the pathogenesis of L. akiyami A from Uthaidhanee of Central Area to compare the pathological changes with L. akiyami A of Pitsanulok Province.

Material and Methods

We used the same technics as reported in our progress report No. III. The results of our studies were as follows:

I. Leptospira javanica (Bangkok)

Culture and Serology

Sacrificed after	Hemoculture (heart blood)	Liver	Kidney	Serology Titre
24 hrs.	+	+	+	0
48 hrs.	+	+	+	0
72 hrs.	+	+	+	0
96 hrs.	+	+	+	0
120 hrs.	+	+	+	0

After 144 hours the remaining animals died of leptospirosis, and the cultivations were all contaminated.

Histopathology

Heart After 24, 48, 72, 96 and 120 hours, slight edema and congestion were seen. There were hemorrhages between muscle fibres in only one animal died of leptospirosis after 144 hours.

Liver After 24 hours, liver architecture was disrupted and the liver cells were swollen.

After 48 hours, edema, congestion and scattered cellular infiltration (most cells were mononuclear cells) were noted.

After 72, 96, 120 hours. only edema and congestion were noted.

Marked congestion and edema, focal necrosis and cellular infiltration, presence of acidophilic cells and Kupffer cells proliferation were noted in one animal died of the disease after 144 hours.

Kidney After 24, 48 hours, edema, congestion, hydropic degeneration of the epithelial lining of the tubules with few hyaline cast were noted.

After 72, 96, 120 hours, atrophy and disappearance of tubular elements of which were replaced by edematous connective tissue were noted. Numerous tubules occluded by casts were also noted.

The findings compatible with lower nephron nephrosis were noted in three animals died of the disease after 144 and 168 hours.

Lungs After 24, 48, 72, 96 and 120 hours, only edema and congestion were noted.

Interstitial pneumonitis was noted in three animals died of the disease after 144 and 168 hours.

Conclusion: Leptospira javanica was virulent to hamsters. Histopathological changes of the vital organs were not remarkable. These findings may confirm the findings in human cases suffering from L. javanica of Bangkok and Chiangmai that most cases were considered to have mild clinical manifestations.

II. Leptospira icterohemorrhagiae (Uttaradith strain)

Culture and Serology

Sacrificed after	Hemoculture (heart blood)	Liver	Kidney	Serology reciprocal titre
24 hrs.	+	+	+	0
48 hrs.	+	+	+	0
72 hrs.	+	+	+	0
96 hrs.	+	+	+	0
120 hrs.	+	+	+	30

Gross hemorrhage The hemorrhages were found only in the lungs after 24 hours in every sacrificed animal throughout the experimental period.

Histopathology

Heart After 24, 48, 72 and 96 hours, only slight congestion was seen. There were congestion and hemorrhage in only one animal died of the disease after 120 hours.

Liver After 24 hours, liver architecture was disrupted and the liver cells were swollen.
After 48 hours, the changes were more pronounced with liver cells necrosis and hemorrhages in some areas.
After 72 and 96 hours, there were many areas of liver

cells necrosis and cellular infiltration. Most of the infiltrated cells were lymphocytes and monocytes. After 120 hours, in one animal died of the disease, showed definite liver necrosis with diffuse cellular infiltration. The infiltrated cells were mostly lymphocytes and monocytes.

Kidney

After 24 hours, there were congestion and cloudy swelling of the tubule.

After 48 and 72 hours, there were edema, congestion, and the tubular epithelium were swollen with few blood casts in side.

After 96 hours, the kidney showed edema, congestion, tubular necrosis with hyaline cast in the tubule.

After 120 hours, in the animal died of the disease the findings were almost the same as seen in sacrificed animal after 96 hours.

Lungs

After 24 hours, there were congestion and few areas of hemorrhagic spots. Slight edema of large bronchiole was also seen.

After 48 and 72 hours, there were interstitial pneumonitis in some areas and hemorrhages were seen in many areas.

After 96 hours, the lungs showed definite changes of edema, congestion, interstitial hemorrhage and pneumonitis.

After 120 hours, in the animal died of the disease, marked interstitial hemorrhages and pneumonitis were seen.

Conclusion: *Leptospira icterohemorrhagiae* (Uttaradith strain) was virulent to hamsters. Histopathological changes of the vital organs were remarkable, particularly in the lungs and kidney. If Uttaradith strain is resembling the Chiangmai strain (the same northern areas), we could expect the changes in the lungs of human cases.

III. Leptospira akiyami A (Uthaidhanee strain).

Culture and Serology

Sacrificed after	Hemoculture (heart blood)	Liver	Kidney	Serology reciprocal titre
24 hrs.	+	+	+	0
48 hrs.	+	+	+	0
72 hrs.	+	+	+	0
96 hrs.	+	+	+	0
120 hrs.	+	+	+	0

Gross hemorrhage The haemorrhages were found only in the lungs of every animal sacrificed after 24 hours throughout the experimental period.

Histopathology

Heart After 24, 48, 72, 96 and 120 hours, no definite change of the heart.

Liver After 24 hours, there were congestion, cloudy swelling and scattered cellular infiltration. Most of the cells were neutrophils. The liver cell nuclei were varies in size and shape.

After 48 and 72 hours the changes were almost the same as after 24 hours. There were also cellular infiltration around the central vein.

After 96 hours, the infiltrative cells around the portal area and central vein were mostly mononuclear and plasma cells. Some areas showed hemorrhages and the liver nuclei showed variation in sizes.

After 120 hours, liver cells showed degenerative changes and cells patterns were destroyed.

Kidney

After 24 and 48 hours, only congestion and edema were seen.

After 72 hours, white cell casts and hyaline casts were seen in the tubules. Some areas showed cellular infiltrations with mononuclear cells.

After 96 hours, some areas showed tubular necrosis.

After 120 hours, some areas of hemorrhages were seen, and showed definite tubular necrosis.

Lungs

After 24 and 48 hours, only edema and congestion were seen.

After 72 hours, some areas of interstitial hemorrhages were seen

After 96 hours, interstitial pneumonitis was also seen.

After 120 hours, marked interstitial pneumonitis were seen with many areas of interstitial hemorrhages, particularly in the animal died of the disease after 120 hours.

Conclusion: Leptospira akiyami A (Uthaidhanee strain) was virulent to hamsters. Histopathological changes of the vital organs were not so remarkable as seen in Pitsanulok strain. (in the report No. III). Pitsanulok strain was considered to be very high virulent to human cases of that area.

9. A comparative serological investigation for the use of filter paper as a transport medium

Our preliminary results in the semi-annual report (J-210-7, July 1968) indicated that the dried blood filter papers kept at room temperature showed slight deterioration of leptospiral antibodies during the second week. We have studied many cases more with different serotypes and initial agglutinin titres. The studies were also extended to the studies of hyperimmunized rabbits blood in order to compare the results with human blood. The results are shown in Tables I-XIV.

Table I. Leptospirosis bataviae (human), initial agglutinin titre was 1:300

Days after Keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at + 4°C.	Sera kept at -20°C.
7	300	300	300
14	300	300	300
21	100	300	300
28	100	300	300
35	100	300	300
42	100	300	300
49	100	300	300
56	neg.	100	300
62	neg.	100	300

Table II Leptospirosis bataviae (human), initial agglutinin
titre was 1:1000.

Days after keeping	Reciprocal titres of leptospiral antibodies.		
	Papers kept at room temp.	Sera kept at + 4°C.	Sera kept at -20°C.
7	1000	1000	1000
14	1000	1000	1000
21	1000	1000	1000
28	1000	1000	1000
35	1000	1000	1000
42	1000	1000	1000
49	1000	1000	1000
56	1000	1000	1000
63	300	1000	1000
70	300	1000	1000
77	300	1000	1000
84	100	1000	1000

Table III. Leptospirosis bataviae (human) initial agglutinin titre
was 1:3,000.

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at + 4°C.	Sera kept at - 20°C.
7	3000	3000	3000
14	1000	3000	3000
21	1000	3000	3000
28	1000	3000	3000
35	300	3000	3000
42	300	3000	3000
49	100	3000	3000
56	100	3000	3000
63	neg.	1000	1000
70	neg.	1000	1000

Table IV Leptospirosis javanica (human), initial agglutinin titre
was 1:300.

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept. at room temp.	Sera kept at + 4 C.	Sera kept at -20°C.
7	300	300	300
14	300	300	300
21	300	300	300
28	300	300	300
35	100	300	300
42	100	300	300
49	100	300	300
63	100	300	300
70	neg.	300	300
77	neg.	300	300

Table V. Leptospirosis akiyami A (human), initial agglutinin
titre was 1:300.

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at + 4 C.	Sera kept at -20°C.
7	300	300	300
14	300	300	300
21	300	300	300
28	300	300	300
35	300	300	300
42	300	300	300
49	300	300	300
56	100	300	300
63	100	300	300
70	neg.	300	300

Table VI Leptospirosis australis A(human), initial agglutinin
titre was 1:1000.

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at + 4° C.	Sera kept at -20° C.
7	1000	1000	1000
14	1000	1000	1000
21	1000	1000	1000
23	1000	1000	1000
35	1000	1000	1000
42	1000	1000	1000
49	1000	1000	1000
56	1000	1000	1000
63	1000	1000	1000
70	300	1000	1000
77	300	1000	1000
84	300	1000	1000

Table VII Leptospirosis canicola (human), initial agglutinin
title was 1:300,000.

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at + 4°C.	Sera kept at -20 °C.
7	300,000	300,000	300,000
14	10,000	100,000	300,000
21	10,000	100,000	300,000
28	10,000	100,000	100,000
35	1,000	100,000	100,000
42	1,000	30,000	100,000
49	300	30,000	30,000
56	300	30,000	30,000
63	100	10,000	10,000

Table VIII Leptospirosis bataviae (rabbit), initial agglutinin
titre was 1:10,000

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at + 4°C.	Sera kept at -20°C.
7	10,000	10,000	10,000
14	10,000	10,000	10,000
21	10,000	10,000	10,000
28	10,000	10,000	10,000
35	10,000	10,000	10,000
42	10,000	10,000	10,000
49	10,000	10,000	10,000
56	10,000	10,000	10,000
63	3,000	3,000	3,000
70	3,000	3,000	3,000
77	3,000	3,000	3,000
84	3,000	3,000	3,000
91	3,000	3,000	3,000
98	3,000	3,000	3,000
105	3,000	3,000	3,000
112	3,000	3,000	3,000
119	3,000	3,000	3,000

Table IX *Leptospirosis javanica* (rabbit), initial agglutinin
titre was 1:30,000

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp	Sera kept at + 4°C.	Sera kept at -20°C.
7	30,000	30,000	30,000
14	30,000	30,000	30,000
21	30,000	30,000	30,000
28	10,000	30,000	30,000
35	10,000	30,000	30,000
42	10,000	30,000	30,000
49	10,000	30,000	30,000
56	10,000	30,000	30,000
63	10,000	30,000	30,000
70	10,000	30,000	30,000
77	10,000	30,000	30,000
84	10,000	30,000	30,000

Table X Leptospirosis canicola (rabbit), initial agglutinin
titre was 1:10,000

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at +4°C.	Sera kept at -20°C.
7	10,000	10,000	10,000
14	10,000	10,000	10,000
21	10,000	10,000	10,000
28	3,000	10,000	10,000
35	3,000	10,000	10,000
42	3,000	10,000	10,000
49	1,000	10,000	10,000
56	1,000	10,000	10,000
63	1,000	10,000	10,000
70	1,000	10,000	10,000
77	1,000	10,000	10,000
84	1,000	10,000	10,000
90	1,000	3,000	3,000

Table XI Leptospirosis icterohemorrhagiae (rabbit), initial
agglutinin titre was 1:100,000

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at +4°C.	Sera kept at -20°C.
7	100,000	100,000	100,000
14	100,000	100,000	100,000
21	100,000	100,000	100,000
28	100,000	100,000	100,000
35	100,000	100,000	100,000
42	100,000	100,000	100,000
49	100,000	100,000	100,000
56	100,000	100,000	100,000
63	30,000	100,000	100,000
70	30,000	100,000	100,000
77	30,000	100,000	100,000
84	30,000	100,000	100,000
91	30,000	100,000	100,000
98	30,000	100,000	100,000
105	30,000	100,000	100,000
112	30,000	100,000	100,000
119	3,000	3,000	10,000

Table XII Leptospirosis hebdomadis (rabbit), initial agglutinin
titre was 1:10,000

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at +4°C.	Sera kept at -20°C.
7	10,000	10,000	10,000
14	10,000	10,000	10,000
21	10,000	10,000	10,000
28	3,000	10,000	10,000
35	3,000	10,000	10,000
42	3,000	10,000	10,000
49	3,000	10,000	10,000
56	3,000	10,000	10,000
63	3,000	10,000	10,000
70	3,000	10,000	10,000
77	3,000	10,000	10,000
84	3,000	10,000	10,000
91	3,000	10,000	10,000

Table XIII

Leptospirosis grippotyphosa (rabbit), initial
agglutinin titre was 1:3,000

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at +4°C.	Sera kept at -20°C.
7	3,000	3,000	3,000
14	3,000	3,000	3,000
21	3,000	3,000	3,000
28	3,000	3,000	3,000
35	3,000	3,000	3,000
42	3,000	3,000	3,000
49	3,000	3,000	3,000
56	3,000	3,000	3,000
63	3,000	3,000	3,000
70	3,000	3,000	3,000
77	3,000	3,000	3,000
84	3,000	3,000	3,000
91	3,000	3,000	3,000
98	1,000	3,000	3,000
105	1,000	3,000	3,000
112	1,000	3,000	3,000
119	300	1,000	1,000

Table XIV. **Leptospirosis wolffii (rabbit), initial agglutinin**
titre was 1:10,000

Days after keeping	Reciprocal Titres of leptospiral antibodies		
	Papers kept at room temp.	Sera kept at +4°C.	Sera kept at -20°C.
7	10,000	10,000	10,000
14	10,000	10,000	10,000
21	3,000	10,000	10,000
28	3,000	10,000	10,000
35	3,000	10,000	10,000
42	1,000	3,000	10,000
49	1,000	3,000	10,000
56	1,000	3,000	10,000
63	1,000	3,000	10,000

Discussion and Conclusion:

From our studies in dried blood on filter papers of 10 cases of human leptospirosis (suffering from six different serogroups), the results indicated that leptospiral antibodies showed no change with in the first week when the papers were kept at room temperature. Four out of ten, however, slightly deteriorated by the end of second week.

The other six specimens showed slight deterioration by the end of third, fourth, fifth, eighth, ninth and tenth week respectively.

The antibodies could be detected up to the third week in only one specimen, the other six specimens were detected up to fifth, seventh, eighth and ninth week. The remainders three lasted longer than ten weeks. Fresh sera kept at $+4^{\circ}\text{C}$, the leptospiral antibodies showed much more stable and lasted much longer than the dried blood.

The studies of seven hyperimmunized rabbits blood, the leptospiral antibodies in dried blood on filter papers showed much more stable than human blood. Fresh sera of experimental rabbits showed a little bit superior to dried blood for the stability of leptospiral antibodies.

It is clear that "the dried blood filter paper method" is reliable and practical for diagnosis of the disease in suspected cases from provincial areas.

10. PATHOGENIC LEPTOSPIRES DISCOVERED FROM THAILAND

In the year 1951, B. Sundharagiati and S. Euspavanich reported four different serotypes of leptospires isolated from human cases in Bangkok Area. (J. Med. Ass. Thailand 34 : 1(1951). The four isolates were sent to WHO/FAO Leptospira Reference Laboratory, Amsterdam, Netherlands for identification. After thorough studies by cross absorption tests, those isolates were identified as

- (1) L. bataviae, (2) L. icterohemorrhagiae (M20), (3) L. rachmat and (4) L. canicola.

During 1963 - 1964, many new serotypes were discovered from human cases of Chiangmai Province, from rats of Pitsanuloke Province, and from dogs of Bangkok Area. Those isolates were also studied at the Amsterdam's Leptospira Reference Laboratory.

The additional new serotypes were:

(5) L. javanica (Veldrat Bat. 46) from man and rats of Chiangmai and Pitsanulok Provinces,

(6) L. akivami Δ from rats of Pitsanulok Province,

(7) L. saxkoebing from rats of Chiangmai Province,

(8) L. bangkok D92 (Australis Δ group) from a dog of Bangkok. It has been proved to be a new serotype and reported elsewhere (Trop. geogr. Med. 17, 20, 1965).

Since 1965 until now, from hemocultures in human cases, kidney cultures from rats of various provinces of Thailand and from kidney cultures of swine, many hundred isolates were discovered.

The followings are additional new serotypes so far discovered in Thailand:

- (9) L. pyrogenes from human cases of Bangkok and rats of Choburi Province.
- (10) L. pomona from swine of Bangkok's slaughter house.
- (11) L. hyos from rats of Nakorn Nayok Province.
- (12) L. sentot from rats of Nakorn Nayok Province.
- (13) L. lora from rats of Khonkaen Province
- (14) L. grippotyphosa from rats of Choburi Province
- (15) L. hebdomadis from human cases of Choburi Province and from rats of Nakorn Nayok Province.
- (16) L. icterohemorrhagiae (Mankarso) from rat of Choburi Province.
- (17) Icterohemorrhagiae (Wijnberg) from rats of Ranong Province.
- (18) L. wolffii from rats of Nakorn Nayok,
- (19) L. schuiffneri from rats of Ranong Province and
- (20) L. CB377, suspected to be a new serotype, because the result of screening agglutination tests with various antisera showed no definite affinity. Cross absorption tests are being studied at Amsterdam's Leptospira Reference Laboratory.

Conclusion:

There were at least 20 serotypes of leptospirae so far discovered in Thailand.

Those leptospirae were classified as belonged to 12 serogroups, as compare with 14 serogroups of leptospirae throughout the world.

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13. ABSTRACT Epidemiological study of suspected cases of leptospirosis in 39 provincial hospitals in Thailand during January 1968 - December 1968 revealed 197 positives out of 1,377 cases (14.3%). Common Serogroups were <u>L.icterohemorrhagiae</u> , <u>L.autumnalis</u> and <u>L.wolffii</u> . However, in Bangkok Hospitals leptospirosis <u>bataviae</u> was common. Annual variation of human leptospirosis in Thailand (residual antibodies survey) was studied for the third consecutive year and the results indicated that the incidence was slightly increased (from 4% to be 6% and 9% respectively.) Surveys of leptospiral antibodies in Umong Canton (an endemic area near Chiangmai Province) revealed that the area was still considered to be an endemic area of leptospirosis. The correlation studies at Pitsanuloke Province enabled us to find out an endemic area and revealed five leptospiral serogroups were prevalent in Pitsanuloke Province. The dried blood filter paper method was experimentally studied and the results revealed that it was reliable and practical for diagnosis of the disease in suspected cases from provincial areas. In animal experiments, <u>L.javanica</u> (Bangkok) <u>L.icterohemorrhagiae</u> (Uttaradith) and <u>L.akiyami A</u> (Uthaidance) were all proved to be of high virulence to hamsters. There were at least 20 serotypes of pathogenic leptospirae so far discovered in Thailand and belong to 12 serogroups. (Author)			

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